VON HIPPEL-LINDAU DISEASE: A Model Project

Prevention and preventive intervention in a hereditary tumor syndrome

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<u>Translator's Note:</u> This translation is intended as a companion to, not a replacement for, the German original. The original tables and full-color plate should be read along with the translation notes included here.

<page> numbers in the text indicate page numbers in the original German edition.

<page 2>

This book was submitted as a manuscript in competition for the Hufeland prize on the 10th of March 1998. On the 30th of November this work as awarded the Hufeland Prize, a prize donated by German Health Insurance companies. Since a German language modern monograph on von Hippel-Lindau disease did not exist, it was decided to publish this monograph and make it generally accessible. The embargo required prior to the actual awarding of the prize has now been lifted, and final editing for the book has been arranged.

The overall favorable results of the prevention project should and must not downplay the seriousness of the potential course of this disease. Indeed diagnosis of VHL is still frequently made too late, treatment is often too late or too radical, and the prognosis therefore can still be unfavorable.

[engraving] Christoph Wilhelm Hufeland 1762-1836 Personal physician to King Friedrich Wilhelm III and Queen Luise of Prussia. Author of the "Makrobiotik, or the Art of prolonging human life"

Table of contents

	German	
VON HIPPEL-LINDAU DISEASE: A Model Project1	1.	1
Table of contents Foreword		
Von Hippel-Lindau disease – a model?		
Summary Clinical and genetic bases		
History		
Methodology		
Literature analysis		
Epidemiology		
Clinical examination program		
Moleculogenetic Preventive-Diagnosis – Groundwork and Results		
Germline Mutations – Findings		
Information Exchange		
Indications for molecular analysis of the VHL gene		
Angiomatosis retinae		
Registry		
Precautionary Examinations		
Haemangioblastoma of the CNS		
Registry		
Renal changes		
Preventive Medical Aspects		
Phaeochromocytoma		
Preventive Medical aspects		
Adrenal-sparing Phaeochromocytoma surgery, Laparoscopic operation		
Phaeochromocytoma as a unique VHL manifestation		
Pancreatic changes		
1. Cystic Pancreatic changes		
2. Islet cell tumors		
Epididymal cystadenoma		
Inner Ear Tumors (endolymphatic sac tumor)		
Prevention of recurrences.		
Genotype/Phenotype correlations		
Cost analysis		
Legal aspects		
National Networking		
Information service		
Balance sheet and perspectives		
Balance sheet		
Perspectives		
New developments in the expansion of diagnoses		
Registry		27
Additional aspects of the Project		
References	71 .	

Figures

German English Figure 1: Common lesions of VHL disease Figure 2: Age at manifestation of individual organ lesions in cases of VHL 12 8 Figure 3: Registered families with VHL-disease 14 8 Figure 4: Distribution of VHL in Germany.

Figure 5: Diagram of Chromosome 3	17 .	9
Figure 6: SSCP from PCR products of Exon 3.		
Figure 7: SSCP (A) and Sequencing (B)	18.	10
Figure 8: Family tree of a VHL family	19.	10
Figure 9: Classical retinal angioma	24 .	12
Figure 10: Histological results of a retinal angioma	25 .	12
Figure 11: Microangioma of the retina in the vicinity of the macula	25 .	12
Figure 12: Fibrous Angioma	25 .	12
Figure 13: Angioma in the vicinity of large vessels	26 .	13
Figure 14: Angioma in the vicinity of the Optic Nerve	26 .	13
Figure 15: Fluorescein Angiograms	27 .	13
Figure 16: Histology of a haemangioblastoma	30.	14
Figure 17: Cystic Cerebellar Haemangioblastomas	31 .	14
Figure 18: Haemangioblastoma of the cervical cord	32 .	14
Figure 19: Observed Progress of tumor growth	35 .	15
Figure 20: Course of a haemangioblastoma in a 22-year-old patient	36 .	15
Figure 21: Bilateral renal changes	37 .	16
Figure 22: Clear-cell, microcystic renal carcinoma in VHL	38 .	16
Figure 23: Prognoses of VHL-associated RCC compared to sporadic RCC	39 .	16
Figure 24: Presymptomatic renal findings in a 27-year-old patient	40.	17
Figure 25: Asymptomatic 30 year old VHL patient	43 .	17
Figure 26: 42-year-old Renal Patient	45 .	18
Figure 27: Diagnosis of Phaeochromocytoma	46 .	18
Figure 28: Precautionary findings in a 30-year-old VHL patient	49 .	19
Figure 29: Abdominal scars from adrenal surgery	52 .	20
Figure 30: Multiple Pancreatic cysts in VHL disease	54 .	21
Figure 31: Serous cystic adenoma of the Pancreas	55 .	21
Figure 32: Islet cell tumor in a 20-year-old patient	55 .	21
Figure 33: Testing for Epididymal cystadenoma	56 .	21
Figure 34: Location of the "Endolymphatic Sac"		
Figure 35: Penetrance –Calculations for mutation VHL nt. 505 T→C	63 .	23

Tables

German English

		ignon
Table 1: Target organs with frequent and unusual manifestations of VHL-disease	9	6
Table 2: Germline mutations of VHL gene in asymptomatic individuals	20	10
Table 3: Germline mutations in patients with retinal Angiomas		13
Table 4: Germline mutations in patients with Haemangioblastomas of the CNS		15
Table 5: Asymptomatic Haemangioblastomas of the CNS		15
Table 6: there is no table 6 in the original		
Table 7: Germline mutations in VHL-associated Renal carcinoma	41	17
Table 8: Organ-saving treatments for VHL-associated renal carcinomas	42	17
Table 9: Sensitivity and Specificity of tests for Phaeochromocytoma	47	18
Table 10: Germline of VHL gene in patients with phaemochromocytomas.		
Table 11: Newly detected Phaeochromocytomas in VHL families		
Table 12: Germline mutations in children with Phaeochromocytoma	53	20
Table 13: there is no table 13 in the original		
Table 14: Germline mutations and associated lesions		
Table 15: Findings and risk profile for patients with germline mutation nt. 479 T \rightarrow C		
Table 16: Findings and risk profile for patients with germline mutation nt. 775 C \rightarrow T		

<page 4> Foreword

The project of "von Hippel-Lindau disease" (VHL) has been planned and worked on for 15 years. In this paper the results through March 1998 have been consolidated, and the results reported here are based on this data. Understanding fully the seriousness of the disease, the author has from the beginning set as his highest priority improvement in the treatment and consequently the prognosis for individuals with VHL.

The complexity of this project makes it clear that only through extraordinarily good interdisciplinary cooperation would it be possible to attain the long-awaited desired results. The project has been continuously supported by the medical directors and has had the cooperation of the chief physicians of the various divisions of the Freiburg University Clinics, especially the Eye Clinic, the Neurosurgical Clinic, the Radiological Clinic, the Surgical Clinic, the Urological Clinic and the Pathological Institute. Many colleagues outside the Freiburg University have actively supported the project and the preventive aspects of this project have thus been broadened and strengthened. To this team of authors belong Dr. Bender, Dr.Natt, Dr.Apel, Frau Müller, Frau Stick, Frau Reifsteck, Frau Heine and doctoral students Mr.Lucas, Mr.Gutsche and Mr.Gläsker. All collaborators who have supported this Project deserve my special thanks. I thank Mr. Munk for his assistance in preparing the manuscript for this book.

<page 5> Von Hippel-Lindau disease – a model?

Von Hippel-Lindau disease is one of the few hereditary illnesses in which an early diagnosis offers favorable therapeutic prospects. It belongs among the group of hereditary tumor conditions. Prevention therefore first of all involves diagnosis of the affect-carrier status in order to study the person at risk and treat any tumors, before irreversible local or systemic damage can occur. Until 1993 the diagnosis of VHL could only be made clinically. After discovery of the VHL gene, a molecular genetic diagnosis of the affect-carrier members of VHL families has become possible. This is similar to other hereditary neoplasms whose basic pathogenetics have been clarified, for example Colon carcinoma (APC-gene, HNPCC-gene), Mammary carcinoma (BRCA1 gene etc.), Multiple Endocrine Neoplasias Type 1 and t=Type 2 (MEN1 gene, RET gene), Papillary renal carcinoma (MET gene), and others. The experiences with these diseases is however quite variable, due first to the complex structure of the gene, and then also to problematic practical circumstances. The unusual features which the prevention project has encountered consisted first of all in the evaluation of the anomaly (site of the genetic mutation) and the illness, and second in the numerous manifestations of VHL.

This project as conceived is a good candidate for a model project because of the multidisciplinary challenges as well as the cooperative integration of molecular genetics into a clinical care program.

The project has received foreign attention and was of orientational help in the structuring of VHL studies in France, in the Netherlands, in Poland, as well as in Japan.

<page 6> Summary

The Von Hippel-Lindau Syndrome (VHL) is a hereditary condition with autosomal dominance. The penetrance is high, the expression quite variable. Numerous organs and organ systems are at risk of formation of tumors, which occur predominantly during the second through the fourth decades of life. Issues generally include retinal angiomas, haemangioblastomas of the cerebellum, the brain stem and the spinal cord, renal carcinomas and phaeochromocytomas. The life expectancies are reduced in retrospective analyses by about 10-15 years. With timely diagnoses, the manifestations are very easily detected during a routine surgical, neurosurgical or ophthalmologic therapy.

Preventive medicine is of primary importance in VHL. It is essential to establish the diagnosis, to avoid complications by early screening examinations of patients with possible lesions, and following the establishment of the diagnosis to offer screening also to their family members. The recently available molecular-genetic (DNA) analysis of the VHL gene offers a new method for the identification or exclusion

of the affected members of a family. If the affect is present, the risk profile for a particular mutation can be calculated through the study of Genotype/Phenotype correlations.

The present Project, initiated in 1983, is based on about 1100 patients with suspect lesions, in which the diagnosis of VHL appeared in a total of 151 families, among which 327 affected persons were obtained. Through DNA analysis the status of 810 patients and their relatives were determined. 56 different germline mutations were found. 10 of these mutations had not been described previously. Affect-carriers were studied following a standardized program. A great number of tumors were found in asymptomatic or symptomatic but readily treatable stages, and were treated accordingly. Altogether 40 operations were performed on haemangioblastomas of the CNS, 17 organ-sparing renal surgical procedure treatments, 41 phaeochromocytoma resections, 2 Whipple operations because of Islet-cell tumors of the pancreas, and 3 resections of inner ear tumors. In addition, Laser treatments of 44 eyes were carried out. These successful treatments are in contrast to 2 neurosurgical complications (incomplete pareses), one postoperative Addison-disease and one case of extensive liver necrosis following Islet cell tumor removal. Through treatment or lack of treatment cpage 7> during the course of the Project, there have been no new cases of blindness, no deaths caused by an operation, no metastasis from renal carcinomas, and no dialysis failure. Some patients with asymptomatic lesions, weighing the risks of a procedure and hoping for a spontaneous improvement, often chose to wait before following the course of treatment recommended.

Throughout the project organ-sparing operations and minimally invasive forms of procedures were used whenever possible in the treatment of VHL. These were often designed specifically for the patients. These included kidney-sparing surgery for multiple renal carcinomas, adrenal-sparing surgery for Phaeochromocytomas, and laparoscopic resections for Phaeochromocytomas.

An essential part of this Project has been the scientific inquiry on a number of detail questions, to obtain better information to advise the patients and their health care teams. As a result, a general information document was compiled and confidential patient information was preserved. This has led to broad cooperation within Germany and the opportunity to include a larger number of patients in regular "controls" or imaging studies to check for recurrent or additional lesions. These controls served to reassure the VHL patients with respect to their lifetime risks and to ensure early detection of recurrence. Altogether 27 recurring tumors in eyes, CNS and Adrenals were detected and successfully treated.

The Freiburg University Clinic, in which the author is active, has been a treatment or advisory center for patients from home and abroad as well as from overseas. Through modern communication media there exists a close collaboration with involved colleagues, with the newly organized VHL Clinical Centers, as well as with various Self-Help groups, especially with the VHL Family Alliance in the USA, on whose Medical Advisory Board the author has served since 1993.

<page 8> Clinical and genetic bases

VHL is a hereditary tumor condition. The inheritance is autosomal-dominant. The penetrance is high, though variable for individual lesion. The most frequent lesions are shown in Figure 1.

Figure 1: <page 8> Common lesions of VHL disease

Angiomatosis retinae – Haemangioblastoma of CNS – Phaeochromocytoma – Pancreatic Cysts -- Renal Cysts and Renal Carcinoma

Furthermore a great many unusual variations in the VHL have been observed, usually in the form of cysts, adenomas or angiomas in the parenchymatous organs or as endocrine neoplasms (3, 51).

The genetic basis was clarified in 1993 with the identification of the VHL gene and by the germline mutation of this gene explaining the origin of the disease (52). The VHL gene appears to be a tumor-suppresor gene, located on the short arm of the third chromosome (3p25-26). (27)

The frequent and rarer changes are summarized in Table 1.

Table 1: <page 9=""> Target organs with frequent and unusual manifestations of VHL-organized</page>	lisease
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Eye	Retinal angioma ¹ Haemangioblastoma (46)	48%
CNS	Haemangioblastoma ¹ Astrocytoma Papilloma of Choroid Plexus (38) Ependymona (47) Neuroblastoma (49)	52% 0.3%
Kidney	Renal carcinoma ¹ Renal cyst ¹	22% 33%
Pancreas	Multiple cysts ¹ Serous Cystic adenoma Islet cell tumor Haemangioblastoma (51) Adenocarcinoma (51)	22% 1.0% 1.0%
Adrenal + Paraganglia	Phaeochromocytoma ¹ (adrenal & extra-adrenal) Haemangioblastoma (40)	29%
Hypophysis APUD cells Epididymus Testes Mesosalpinx Liver	Adenoma Carcinoma Cystic adenoma ¹ and cysts (in males) Germ cell tumor (41) Cystic adenoma (44,50) Cysts Angiorga Adenoma Cargingma (45,47,51)	0.3% 1.0% 8% 1.0%
Spleen	Angioma, Adenoma, Carcinoma (45,47,51) Cysts Angioma (47)	0.3%
	Cysts, Angioma (47) Cysts (39) Angioma Angioblastoma (47) Endolymphatic sac tumor <i>ntages are based on the appearances in individual healthy pati</i> <i>script = Classical lesions, basis for clinical diagnosis, see text.</i>	

<page 10> Diagnostic criteria were obtained from Clinics and genetics. These have been grouped at the present time into three different forms of minimal criteria (3,54,57):

1. A patient with retinal angioma or Haemangioblastoma of the CNS as well as a relative with a lesion such as listed in Table 1. These criteria cover the majority of patients.

2. A patient with retinal angioma or Haemangioblastoma of the CNS and one other classical lesion as described in Table 1. These cases as a rule make up new mutations.

3. A patient with only one of the classical lesions as described and the molecular-genetic detection of a mutation in the VHL gene.

<page 10> History

The disease is named for two physicians, Eugen von Hippel and Arvid Lindau. Eugen von Hippel was an Ophthalmologist in Heidelberg and Göttingen and wrote in 1904 "About a very rare disease of the retina" and furnished in 1911 "The anatomical basis of the very rare disease of the retina described by me." (59,60) Arvid Lindau was a pathologist in Stockholm and Lund and published in 1926 his studies "about Cerebellar cysts – Pathogenesis and relationships to Angiomatosis retinae" (53).

Both authors furnished classical descriptions and interpretations. The first to describe a family with VHL however was Collins (1894) (48), the first to describe retinal angioma was Deval 1862 (43); first to describe cerebellar Haemangioblastomas was Jackson (1872) (48).

<page 11> Methodology

In this section the composition of the Project, its components as well as its methodological bases are described. In the following sections, details will be combined with results and discussion.

An analysis of the literature provided the basis for diagnosis of VHL (see p.10), for age of manifestions, and for prognosis. This provided the starting point for improvement in care and management

Epidemiological studies of regions provided evaluation of the prevalence of VHL. Prior experience provided a starting point for treatment of VHL and its individual lesions.

The clinical research protocol had to be standardized, fitted into an amount of time suitable for the clinic and the patient, and methods found that would be the least invasive and financially justifiable.

Molecular-genetic (DNA) diagnosis was introduced into our Project in 1994. It is based on established techniques. It was used to clarify the indications of the clinical diagnosis, as well as to study genotype/phenotype alignment and to assist in assessing prognosis.

As a model for preventive medicine the Project set out to determine whether it would be helpful to form a network of satellite centers in order to support patients outside the geographic reach of Freiburg.

Because management of VHL requires an extensive flow of information, it was considered whether special services would be needed and would be well accepted. Various forms of communication were established, for example informational meetings in which the patients participate, Information Centers, Self-help groups and the use of Networks.

Early detection, approaches to therapy, and recorded outcomes have been assembled into groups for individual lesions.

Genotype/Phenotype Correlations serve to evaluate the specific risk factors of the Prognosis.

Finally the Balance sheet is drawn up, in which the various actual problem conditions are explained and the prospects for improvement in management are shown.

The Cost Analysis was necessary for orientation and illustrates the actual state of initial and followup studies.

<page 12> Literature analysis

An analysis of the literature demonstrates that somewhat more than 1000 publications about VHL exist. The data on manifestations, therapy, complications and prognoses are very heterogeneous. There are individual cases and family recollections, studies of pathoanatomic or clinical views as well as a series of organ manifestations such as retinal angiomas. The 337 cases included in this Project show that VHL first arises chiefly in adolescence and early adulthood (2) (see Figure 2).

Figure 2: <page 12> Age at manifestation of individual organ lesions in cases of VHL Cumulated age distribution of lesions of the eye, CNS, kidneys and adrenals among 337 patients with VHL (2)

The spectrum is quite large and extends from 4 to over 80 years. Retinal angiomas were symptomatic earlier than tumors of the CNS, Phaeochromocytomas and Kidney lesions. The lesion spectrum was quite variable, whereas in the section Genotype/Phenotype Correlation it is more contracted. <page 13> A large meta-analysis shows that retinal angiomas occur in about 57%, Haemangioblastomas in about 61%, Renal changes in about 32%, Phaeochromocystomas in about 19%, Pancreatic tumors in about 4% and Epididymal cystadenomas in about 17% (51).

The prognosis was usually obtained in the form of a cause of death analysis. The leading causes of death were Haemangioblastomas of the CNS and metastatic Renal carcinomas, more rarely Phaeochromocytomas, Islet cell tumors of the Pancreas or other lesions (3. 51,55). The life-expectancies ranged about 15-20 years lower than the general population (55). These data were obtained through retrospective analyses, that is modern diagnostics and therapies were not yet available for these patients. The Literature Analysis showed that VHL

- 1. is accompanied by higher morbidity and mortality.
- 2. is manifested predominantly in the 2^{nd} through 4^{th} decades of life.
- 3. consists of diagnostic and therapeutic available components.

<page 14> Epidemiology

The primary manifestations of VHL are an appropriate basis for epidemiology studies. The majority of these lesions are unusual and their therapies require institutions with large clinics (for example, a neurosurgical clinic). Following construction of appropriate registries for retinal angiomas, Haemangioblastomas of the CNS and phaeochromocytomas, for the first time in the circumscribed geographic region of South Baden with 1.9 million inhabitants, a prevalence of VHL could be calculated by the author as about 1 : 39000 inhabitants (4). Shortly after this, a census was published for Middle England, which estimated a similarly high prevalence of 1 : 35000 (54). These data were obtained from clinical studies. Over- and under-estimates are possible. For one thing in an under-studied area there are VHL families who no longer know that they are related to one another (the so-called Founder Effect), or cases of affect-carriers exhibiting atypical manifestations (i.e. patients with only a Phaeochromocytoma, but who are carriers of a germline mutation of the VHL gene). Both are possible, but there are currently no studies which include all these possibilities. The dynamics of developing this Project allowed us to gather the number of identified VHL families in South Baden. Until 1993 there were no clinical compilations. Following the introduction of DNA diagnosis the surge in demand increased, which led to the identification of 150 VHL families in Germany. (See Figure 3.)

Figure 3: <page 14> Registered families with VHL-disease

(Project finding since 1983) Development of registers – number of Families Bar graph shows the growth of the number of identified families over the course of the Project <pr

Figure 4: <page 15> Distribution of VHL in Germany.

The dots on the map indicate the residences of involved families. Each dot may represent one patient as well as one family.

<page 16> Clinical examination program

The clinical examination program since 1983 has undergone several revisions, following the evolution of radiological techniques. The current standard program consists of:

- 1. Magnetic Resonance Imaging of Head with Gadolinium as Contrast medium.
- 2. MRI of Spinal Cord with Gadolinium as contrast medium.
- 3. MRI of Abdomen without Contrast medium
- 4. Ophthalmologic examination (Retinoscope)
- 5. Sonograph examination of testes
- 6. Catecholamine determination by 24-hour Urine
- 7. Preparation of EDTC-Blood for molecular-genetic Diagnosis.

MRI with Gadolinium is now the standard for Haemangioblastomas of the CNS. Computer-Assisted Tomography (CAT) of the head is obtained using a contrast medium a similar high sensitivity and specificity, but it is radiation. This method of course is undesirable for the spinal cord. The MRI of the head and spinal cord with modern equipment and software programs can be carried out in about 30 minutes. With a good picture of the inner ear region, it is then possible to evaluate inner ear tumors as well.

For the examination of the kidneys, adrenals and pancreas, the CAT-scan with contrast medium is the standard today. Small Phaeochromocytomas are better detected in T2-density MRI's, and pancreatic and renal lesions are well depicted using modern MRI equipment. We use them to avoid radiation exposure. The use of a contrast medium has not been necessary in our experiences thus far. Sonography of the Abdomen is dependable when obtained from our trained staff, though with some sound limitations, especially for the Pancreas. Left-sided, small and extra-adrenal Phaeochromocytoma were rarely overlooked (12, 15). This method is also suitable for monitoring the course of known lesions. The Eye examination should be performed in mydriasis (full dilatation of pupil) and for finding peripheral angiomas with the use of a contact glass (Goldmann's *Dreispiegelkontakglas*). The assessment of Catecholamines by 24-hour-Urine includes the determination of levels of Noradrenalin, Adrenaline and Vanillylmandelic acid. Uncertain or pathological changes could necessitate additional special examinations.

<page 17> Moleculogenetic Preventive-Diagnosis – Groundwork and Results

After about 10 years of intensive research work, the VHL gene was identified in 1993 by a research group headed by the U.S. National Institutes of Health (NIH) (52). This research was carried out by several teams from various countries. A series of clinics whose family studies formed the basis for the gene identification was involved. The author had met in Boston the molecular-genetic research group that was able to narrow down the gene location to Chromosome 3p25-26, (27), even though the gene itself had not yet been identified (see Figure 5).

The VHL Gene consists of a promoter area, of 3 exons and 2 introns. The code sequence consists of 852 nucleotides and codes for a specific protein (pVHL) of 284 aminoacids (see Figure 6).

Figure 5: <page 17> Diagram of Chromosome 3

with localization of VHL gene telomere with the short arm in the band region 3p25-26.

Probe G#35 shows a normal band pattern. The Probes G#773, G#2111 and G#697 show aberrant band patterns. The significance of the sequencing has yet to be interpreted.

<page 18> The molecular-genetic analysis of the gene includes the following steps:

- 1. DNA-Denaturing
- 2. Amplification of exons as well as exon-segments with specific primers
- 3. Single stranded conformational polymorphism (SSCP: Polyacrylimide-gene-Electrophoresis of denatured individual strands)
- 4. Sequencing

Figure 7: <page 18> SSCP (A) and Sequencing (B)

A: Trace 1 (N) shows a normal control, trace 2 (+) shows the aberrant band of a VHL patient, and the traces 3 and 4 show normal finding in the parent. The finding reveals a new mutation. B: Identification of germline mutation nt. 490 G – A (Gly 93 Ser). The arrows indicate the difference in comparison to the Wildtype sequence (from 35).

<page 19> The Primary Pair used for analysis are two pairs in Exon 1 two pairs and one each for Exons 2 and three. In the SSCP, in order to avoid radioactive exposure of laboratories and personnel, a special silver dye with very good sensitivity is used (Fig. 6 and 7A).

Figure 7 shows an example of a SSCP and the pertinent sequencing. In the examination of familial relationship, in case of a good clear Band aberration in the SSCP there may be omissions in the sequencing. In family examinations a restriction-enzyme test may be used as an alternative for the SSCP.

Thus after PCR of Exon 1 with a modified primer pair the mutation nt. 505 C \rightarrow T can be proven by the enzyme Fok I. The mutant DNA will separate and appear as two bands, while the normal DNA will not separate and only one band will appear (see Figure 8).

Figure 8: <page 19> Family tree of a VHL family

and examination of 13 family members with a specific restriction enzyme test (Fok I digest of PCR-amplified Exon 1). Explanation of Predisposition -- VHL family with Angiomatosis retinae and Phaeochromocytoma - - Restriction-enzyme test

In the restriction-enzyme test a double band indicates the existence of a mutation (VHL nt. 505 T \rightarrow C). This test shows that persons 10 and 12 have normal findings. For persons 11 and 13 there is a germline mutation. In the family pedigree retinal angiomas are indicated on the upper left, Phaeochromocytomas on the lower right.

<page 20>

Table 2 : <page 20> Germline mutations of VHL gene in asymptomatic individualsColumn headings: Nucleotide changes - Effect on coding sequence - MutationNomenclature - Mutation type - Index casesColumn content notes:Column 1: C=Cytosine, T=Thiamin, A=Adenine, G=Guanine; kb = KilobasesColumn 2: Amino acids according to the 3-letter designation using the internationalcodes; del = deletion, ins=insertionColumn 3: Amino acid with the one-letter designation using the international code

<page 22> The detection of larger deletions is not possible with the described methods. For this a special Southern blot has been developed, by which deletions between 1kb and 11kb can be detected (see Table 2).

<page 22> Germline Mutations – Findings

In Table 2 are listed all of the mutations obtained up through 1-March-1998 in the laboratory of the author. The number of Index cases shows one patient per family, which according to their knowledge had not changed. Several index cases showed either that the families were undergoing a new mutation, or a so-called Founder Effect, and consequently in fact still could be changed. The Founder Effect could, for example, be indicated for the mutations nt. 505 T \rightarrow C and nt. 775 C \rightarrow G (29, 30).

Altogether 56 different mutations were established, which were found in all three Exons of the VHL gene. There were all types of mutations, that is Nonsense Mutation, Missense Mutation, Intraxonic Deletion or Insertion with or without displacement of Leserasters, Splice site Mutations and large Deletions (1-11 Kilobases). Ten of these mutations have not been previously described in the literature.

In 19 families with clinically ascertained VHL, no germline mutation could be identified by us. No DNA was obtained from 27 families. Proof of the Founder Effect was established in 78% of cases with clinically diagnosed VHL. In addition, molecular-genetic VHL mutations were detected in about 85% of the cases, confirming the diagnosis of VHL. This agrees with international findings (30,31). These "hit rate statistics" are important for setting the level of expectation of a participant as well as the attending physician and the individual patient.

<page 22> Information Exchange

As a practical matter we proceed in the following manner, First we conduct an extensive informational talk with the patient or attending physician, including indications and consequences. In addition we have provided since 1996 on a regular basis a free copy of our Information pamphlet. This is followed by an Analysis. If a laboratory analysis is available, we first share it with them. Thus the patient has the right to this information, unless it is <page 23> his expressed wish not to be informed about the findings; thus the "Right Not to Know" is respected. Thus far none of the VHL patients have made use of this choice not to know. In a personal conversation the results can be imparted and the ensuing consequences discussed.

Because of the great and vital importance of the results we require a second blood sample and conduct a second independent, but methodologically identical, examination in order to confirm the findings. The first findings are considered "preliminary" until confirmed.

<page 23> Indications for molecular analysis of the VHL gene

The following are indications for the genetic examination of the VHL Gene:

- 1. In patients with a clinically diagnosed VHL for confirmation of the diagnosis at the molecular-genetic level (see also section Genotype/Phenotype Correlation),
- 2. Patients with only one lesion out of the complex of VHL to clarify whether this is due to a germline mutation. We recommend this procedure instead of a clinical examination. Candidates are all patients with one of the classical lesions from the spectrum of VHL-associated manifestations, to be sure this includes only clear-cell renal carcinomas and only then, if the patient is below the age of 50 years, after which age additional renal cysts occur or multiples tumors may be present.
- 3. To clarify the carrier status of relatives of the patient with positive germline mutation. In the case of a negative family history both parents should be studied, to determine whether this is a new mutation (Fig. 7).

<page 24> Angiomatosis retinae

Retinal angiomas are reddish vascular tumors, which consist of capillaries. Characteristic is the supply by a pair of vessels whereby the feeder and drainage vessels are distinguished by the snake-like (tortuous) configuration and expanded caliber (Fig. 9). The diagnosis is made clinically. Histologically they resemble the structure of Haemangioblastomas of the CNS. Typical age of manifestation is in adolescence and early adult-hood (see Figure 9).

Figure 9 : <page 24> Classical retinal angioma

Classical retinal angioma (left) with tortuous and caliber-expanded pair of supply vessels. On the right: Appearance following Laser-coagulation in the form of a circular sealing (from 25).

Retinal angiomas may lead to detachment of the retina (*amioto retinae*) and thereby a loss of vision (Figure 10). Prodrome or complaints at the onset of *Amioto* are lacking. Treatment will involve a series of attempts to reattach the retina. The results are rather unsatisfactory. In such Amiotic eyes it is not uncommon for a painful secondary glaucoma to develop. An enucleation at this stage is rarely avoided. Three clinical forms of manifestation of Angiomatosis retinae may be differentiated:

- 1. The classical retinal angioma (see Figure 9)
- 2. The micro-angioma (Figure 11)
- 3. The fibrous angioma (Figure 12)

<page 26>

- 1. Angioma at or in the vicinity of the Macula (Fig.11)
- 2. Angioma in the vicinity of the Optic nerve (Fig.14)
- 3. Angioma in the vicinity of larger vessels (Fig. 13)

For documentation, and whenever the clinical findings are not entirely clear for diagnosis, Fluorescein Angiography is used (see Figure 15). Finally we should mention the rare occurrence of an Angioma within the Optic nerve.

Figure 10: <page 25> Histological results of a retinal angioma with retinal detachment in the outermost periphery; lower right in the picture the ciliary body

Figure 11: <page 25> Microangioma of the retina in the vicinity of the macula

Figure 12: <page 25> Fibrous Angioma

<page 32> Registry

In the course of this Project a Registry for retinal angiomas was created. This has now reached 124 cases. The total number of involved eyes totaled 172. 71 of the cases were revealed primarily by appearance of a visual loss. We were unable to pinpoint the exact time of the vision loss in several cases. Vision loss in one eye was first noticed for one 18-year-old boy during a test for a driver's license. The age for diagnosis for this kind of "symptomatic" retinal angioma would be 7 to 54, with an average of 28 years. Through clinical and genetic examinations a diagnosis of VHL disease could have been made in 80% of

In Table 3 are summarized all the mutations in which retinal angiomas were observed. It shows that all mutation forms (Missense-, Nonsense-, etc.) can predispose to retinal angiomas.

Figure 13 : <page 26> Angioma in the vicinity of large vessels

Figure 14: <page 26> Angioma in the vicinity of the Optic Nerve

. <page 27>

Figure 15: <page 27> Fluorescein Angiograms

At 0 minutes and at 60 minutes the supply of the angiomas is not detectable, while the finding at 30 minutes illustrates that the angioma is being supplied from the upper region of the picture

<page 27> Precautionary Examinations

The precautions consist of clinical and genetic examinations. Retinal Angiomas were detected in 63 eyes in 48 patients in asymptomatic studies, that is without visual disturbances and without retinal detachment. In addition follows:

- Complete examination or reevaluation of patients with VHL disease
- Examination of patients with for a long time only one primary lesion of the VHL complex, through which the new diagnosis of the disease would be made.
- Examination of relatives with VHL disease.
- Presymptomatic genetic screening in families.

<page 28>

Table 3: <page 28> Germline mutations in patients with retinal Angiomas

Germline mutations of VHL Genes in patients with retinal Angiomas (extracted from Table 2) see that table for abbreviations

The 110 angiomas identified include: 30% classical angiomas, 55% Microangiomas and 15% fibrous Angiomas. The positions of the angiomas were 90% in the periphery, 5% in the region of the Macula and 5% in the region of the Optic nerve. The Laser coagulation Therapy consists of a circular sealing page 29> and closing obliteration of the angioma (see Figure 9). The therapy is accomplished in 1 to 3 sessions of outpatient treatments

Altogether, 64 angiomas were treated. No secondary effects occurred. The follow-up observation period averaged 7.2 years (max.14). In this time 6 new microangimos and 5 microangiomas in the area of coagulation were observed, which were in turn successfully coagulated, free of side effects (25).

Fibrous angiomas presented no therapy indications. 16 eyes exhibited such angiomas, which showed no change over an average observation period of about 4 years.

17 of the patients with asymptomatic microangiomas were considered untreatable and were closely watched.

Over an average observation period of 4.5 years no detectable changes were noted, from which it was decided that with angiomas in problematic locations, such as in the vicinity of the macula or the Optic nerve, a waiting attitude is a defensible position.

In summary, 110 asymptomatic angiomas were detected during the current Project. The principle of early therapy by Laser coagulation has proven itself to be effective and free of secondary complications. Because of the large number of incidentally detected lesions, experience has been gained in treating problem cases and in understanding the tendency of these angiomas to grow. During the 15 years running time of the Project, none of the eyes exhibiting asymptomatic angiomas became blind under this method of treatment.

<page 30> Haemangioblastoma of the CNS

Haemangioblastomas of the CNS are tumors which consist of two components: the tumor is made up of numerous capillaries with typical endothelial as well as interstitial cells with foamy cytoplasm, whose origin (Glial cells or Astrocytes) has up till now remained unclear (see Figure 16). The tumors are histologically non-malignant (grade 1 according to WHO). Frequently there are cystic-like cavities which are filled with an amber-colored fluid.

Figure 16: <page 30> Histology of a haemangioblastoma

Histology of a haemangioblastoma with capillaries and interstitial cells with foamy cytoplasm

In the cerebellum (Figure 17), these cystic tumors have been named Lindau Tumors based on the classical description by Lindau. They occur also in the region of the brain stem and spinal cord (Figure 18) as well as in very rare cases in the cerebrum. In the spinal cord the longitudinal running spaces form syrinxes. These fluids contain high concentrations of Erythropoietin; the haemangioblastomas of the CNS are thus the classical example of para-neoplastic Erythropoietin synthesis (17), however Erythropoietin is neither in measurable activity parameter in the serum, nor is polyglobulin a constant symptom or a concomitant finding (26).

<page 31>

Figure 17: <page 31> Cystic Cerebellar Haemangioblastomas

with firm-walled solid portion (white arrows) and larger Tumor cyst (dark arrows) a: coronal, b: transverse projection. MRI with contrast medium (Gadolinium). 28 year old daughter of a VHL patient, symptoms at 4 weeks.

Haemangioblastomas of the CNS produce symptoms, due to their localization and their space crowding, such as ataxia, disturbances of gait and other cerebellar signs or radicular deficiencies, as well as brain pressure with headaches, nausea and vomiting. Retrospective studies show that haemangioblastomas in the CNS lead to higher morbidity and mortality (3,51,55). Modern Radiology has made enormous improvement of diagnosis by means of MRI (Figures 17-19). The tumors may be enhanced with Gadolinium as the contrast medium, whereby a T1-loaded photographic technique must be used. The MRI in this way takes pictures in 3 planes, excellently suited for examination of the spinal cord. A sagittal MRI series belongs in a routine examination of every VHL patient.

<Page 32>

Figure 18: <page 32> Haemangioblastoma of the cervical cord

Haemangioblastoma of the cervical cord with round tumors and elongated syrinx.. MRI with contrast medium (Gadolinium), sagittal cut.

<page 26> Registry

In the course of this project a total of 257 patients with Haemangioblastomas of the CNS were registered. This series included 130 patients, who came to Freiburg University Clinic for diagnosis and treatment, as well as 127 who were treated in other clinics. In order to determine the portions of VHL-associated and sporadic haemangioblastomas of the CNS, the clinical examinations of all the haemangioblastomas operated on between 1979 and 1989 at the Freiburg University Clinic were handled by the author and an incidence of VHL-associated cases of about 23% was determined. The comparison of

sporadic and VHL-associated haemangioblastomas showed that VHL-associated tumors occurred about 15 years earlier (5, 10) than sporadic hemangioblastoma. Similarly multiple tumors were found in 50% of patients with VHL, but in only 8% of people with sporadic tumors.

The location occurrence in VHL-associated tumors was 64% intracranial and 36% spinal; in sporadic tumors it was 78% intracranial and 22% spinal.

Table 4 : <page 33> Germline mutations in patients with Haemangioblastomas of the CNS $\,$

Germline mutations of VHL gene among patients with Haemangioblastomas of the CNS (extracted from table 2)

The molecular genetic examination results for Haemangioblastomas of the CNS are collected in Table 4. It shows that all forms of mutation (Missense, Nonsense, etc.) can predispose for Haemangioblastomas of the CNS.

<page 34>

Table 5: <page 34> Asymptomatic Haemangioblastomas of the CNS Asymptomatic Haemangioblastomas of the CNS discovered in the course of this Project. $RM = Spinal \ cord$ Column headings: Case – Age – Sex – Cerebellar/Brain stem – Spinal cord – Number of tumors – Maximum tumor diameter

<page 35> Through genetic and clinical examinations asymptomatic tumor were found in 25 patients (see Table 5). The majority of these tumors were located in the region of the Spinal Cord.

A special problem of this Project was produced by the identification of a large number of asymptomatic Haemangioblastomas. The risk factor of the natural course had to be weighed against the risk factor of an operation. The natural course is characterized by growth, eventually causing compression of the nerve tissue as well as the risk of hemorrhage. Among the total number of the 257 patients registered in the course of the Project with haemangioblastomas of the CNS, a spontaneous bleeding occurred in only one case with a spinal tumor, which led to a partial paraparesis. The growth of the Haemangioblastomas is extraordinarily slow according to our observations. According to our data, only four of the 25 patients with asymptomatic haemangioblastomas decided to have an operation. According to our data and progress monitoring, when symptoms appear in one case, it was found that indeed the tumor had grown over 6 years (from 1-2 mm diameter) into a 3-cm cystic lesion (see Figure 19).

Figure 19: <page 35> Observed Progress of tumor growth

Observed progress of the growth of a primary preliminary finding, prior to operation. Minimal tumor growth (left), and the build-up to cyst after 6 years (right); Haemangioblastoma in a 23-year-old patient, MRI with contrast medium (Gadolinium).

page 36> In keeping with the genetic nature of the disease, there is a life-long risk for new developing tumors. In Figure 20 we show a patient with multiple new tumors occurring at various locations along the CNS. The time of these occurrences serves as supporting evidence for regular monitoring examinations.

Figure 20: page 36> Course of a haemangioblastoma in a 22-year-old patient
Course of development of a haemangioblastoma in a 22-year-old patient with recurring,
newly developing haemangioblastomas

<page 37> Renal changes

Renal changes in VHL are renal cysts and renal carcinomas. Typically these lesions are found in both kidneys. Frequently those kidneys exhibit multiple tumors (see Figure 21).

Figure 21: <page 37> Bilateral renal changes

Bilateral renal changes in a 45 year old VHL patient with cysts and solid, partially cystic tumors.

Histologically they are clear-cell renal carcinomas. As a rule cellular anaplasia is small. Usually the tumors have a connective tissue capsule (Figures 25 and 26). The vascularity is very well developed. Often there are multiple cystic structures within the tumors, which are partially filled with blood (see Figure 22). Metastasis follows haematogenic pathways, chiefly into the lungs, liver and bones. The renal cysts exhibit usually a simple ordinary epithelium and resemble simple cysts. Detailed histological examinations reveal however that in renal cysts there is to be found epithelial proliferation just as in incipient carcinomas. It is however unclear whether in VHL renal carcinomas arise from cystic or non cystic lesions (22, 34).

<page 38>

Figure 22: <page 38> Clear-cell, microcystic renal carcinoma in VHL

Clear-cell, microcystic differently appearing renal carcinoma in VHL Note the abundance of erythrocytes in the lumen of the cysts.

In order to investigate the prevalence of VHL-associated renal tumors, the author had further examined the 460 cases in the clinical and family records of patient care of the Urological Division of the Freiburg University Clinic and established that VHL accounts for 1% of the cases of renal cell carcinoma. This was confirmed through molecular-genetic examinations of 183 (62%), representing all the living patients of this collection.

In order to obtain prognosis criteria, 63 patients with VHL-associated carcinomas were compared to 375 clear-cell sporadic renal carcinomas; the two differed not in respect to the number of tumors, which were detected in the asymptomatic stage and neither with regard to the tumor size. The essential result was that the prognosis of VHL-associated renal carcinomas was significantly better (see Figure 23). In this series, VHL-associated renal carcinoma metastases occurred only with tumors of over 7 cm (22).

<page 39>

Figure 23: cpage 39> Prognoses of VHL-associated RCC compared to sporadic RCC
Prognoses of VHL-associated renal carcinomas (n=63) compared to sporadic renal
carcinomas of the clear-cell type (n=375 from (22). RCC: Renal Carcinoma. The
prognosis in the analysis of the renal carcinoma related deaths is significantly better in
the VHL-associated tumors (upper graph), compared to all deaths from sporadic RCC
(lower graph).

<page 40> Preventive Medical Aspects

For the VHL-associated renal carcinoma, preventive medical procedures are necessary both for the diagnosis as well in the therapy. The early recognition of the renal carcinoma has already been described by clinical and genetic examinations upon persons for whom a risk for this disease exists. In the clinical examinations on affect-carriers, at the present time computed tomography and magnetic resonance imaging have about the same status (see Figure 24). The mutations, which would result in VHL-associated renal carcinomas, are listed again in Table 7. This demonstrates that all the known forms of mutation (Missense-, Nonsense-, etc.) can predispose to renal carcinomas.

Figure 24: cpage 40> Presymptomatic renal findings in a 27-year-old patient
Computer tomography (left) and corresponding MRI (right) both with contrast media:
Multifocal carcinoma and cysts of the left kidney. One can see almost equally well the
reasons for removal.

Preventive medical treatment is the patient's only chance. If the complete kidney is removed for Multifocal and bilateral renal carcinomas, then the patient will have bilateral nephrectomy resulting in chronic hemodialysis, making this asymptomatic person into a patient with a chronic illness. This complex matter was discussed in detail at an international symposium with the participation of the author, Organ-sparing renal tumor surgery is currently the standard practice in VHL disease (34). Differences of opinion exist only about the tumor stage when the operation should be performed. cpage 41> In the United States of America, tumors at 3 cm diameter are considered an indication for surgery. Based on specific results collected in this Project, data was carefully collected and the critical limit set at 5 cm diameter. For the decision to be made properly, one must also consider the anatomical relationship between the tumors and the structures of the hilus, and most importantly the attitude and the willingness of the patient, who must be made aware of the risks associated with his illness.

Table 6: there is no table 6 in the original

Table 7: cpage 41> Germline mutations in VHL-associated Renal carcinoma (extracted from Table 2).

In the course of the Project between 1991 and 1997, organ-sparing renal surgical procedures were performed on 11 patients. In 6 of these patients bilateral operations were necessary. The pertinent data of these patients are included in Table 8. During the operation the kidney was completely exposed, the circulation atraumatically interrupted, the organ superficially cooled. In addition to the large tumors all other risk-free reachable lesions were excised during an ischemic period <page 42> no greater than 90 minutes. For monitoring of progress a Duplex sonograph pre- and post-operatively was found to be suitable (see Figure 25). The results from yearly follow-up examinations have shown no new tumors in these patients. Kidney function, measured by serum creatine, were stable.

Table 8: <page 42> Organ-saving treatments for VHL-associated renal carcinomas

Column headings = Case-age-side-number of tumors-tumor diameter (measurement – cm)-period of observation (in months) – Creatine mg%

The situation is problematic for VHL patients who have already had a unilateral nephrectomy, and who now have multiple tumors in the contralateral kidney with normal kidney functions (see Figure 26). In such patients, the new operation -- especially in cases of multiple extensive tumors – can lead to a definite deterioration of kidney function until the time for dialysis is reached. Two of our patients are in this difficult situation, both of whom have refused an operation on the contralateral kidney and however meanwhile have remained free of metastasis for 3 to 4 years.

<page 43>

Figure 25: <page 43> Asymptomatic 30 year old VHL patient

MRI's, Enucleation preparations and Postoperative Duplex Sonographs of the kidneys with bilateral Phaeochromocytomas and 8 renal carcinomas of the right as well as 9 renal carcinomas in the left kidney; the Duplex Sonography shows a very good blood supply of all renal areas postoperatively, P = Phaeochromocytomas.

VHL Patients on whom a kidney transplant had been performed are extremely rare. In cooperation between the Urological Clinic of Cleveland Foundation and the author a series of 37 such patients world-wide were collected. In comparison with a control study of other kidney transplant recipients, the prognosis of the VHL patients showed no significant differences. Three of the VHL patients died with metastases of renal carcinomas (36).

In summary, the bilateral renal tumors in VHL disease do present one of the central and serious problems for management. If the patient reaches a Center at the right time -- that is, before a renal surgical procedure has been performed -- it is possible that the organ-saving procedure will have at least an average chance of very good results.

<page 45>

Figure 26: <page 45> 42 year old Renal Patient

Condition of the kidney after a right nephrectomy 8 years before (above): multiple carcinomas and cysts in the left kidney; (below): MRI series. The nephrectomy preparation shows only one large tumor as well as smalltumors (two in this cut section) at the apical pole of the organ.

<page 46> Phaeochromocytoma

The central problem of patients with Phaeochromocytomas is the usually very late diagnosis. Arterial Hypertonia is the essential finding; this appears intermittently and therefore will be frequently as "well adjustable" not explained further. Myocardial hypertrophy, left heart decompensation, rhythm disturbances, Myocardial infarct and Cerebral insult are the typical complications. Only a carefully prepared case history will reveal the necessary diagnostic information. Only rarely do the illness and the relatively young age of the patient explain headaches, heart sensations (palpitations or tachycardia and excessive hypertonia) and breaking out in sweat – typical of many occurring symptoms. The symptoms have long been misinterpreted. The spectrum of differential diagnosis is large and extends into psychosis; the trauma experienced during an unnecessary psychosomatic treatment is difficult to imagine.

Figure 27: <page 46> Diagnosis of Phaeochromocytoma

Pictures taken in the course of examinations for diagnosis of Phaeochromocytoma Sonographs (left above), Contrast medium-supported CT (left below), Metaiodobenzylguanidin-scintigram (MIBG) (middle), MRI (right above coronal, right below transverse), P = Phaeochromcytoma, L = Liver, N = Kidney, W = vertebral body,A = Aorta. In the Scintigram the small arrow indicates the Urinary bladder. The large arrow indicates the tumor = T.

<page 47> Preventive Medical Aspects

The preventive medicine for phaeochromocytoma involves three complex questions,

- 1. The adequate diagnosis
- 2. The prevalence of VHL among unselected Phaeochromocytoma Patients
- 3. Early diagnosis and therapy.

1. Diagnosis

Tissue displacement and hormonal activity are the basis for the diagnosis of Phaeochromocytomas. To evaluate the value of the picture-taking procedures Sonography, CAT scan, MRI and MIBG-Scintigraphy (Figure 27) as well as the biochemical analyses of catecholamine in 24-hour Urine and plasma, the author carried out examinations in 25 families with Phaeochromocytomas (15). Phaeochromocytomas were detected in 79 subjects. The subjects went through all of the diagnostic procedures. The results are summarized in Table 9. Since that time the Project has used the combination of MRI's and Catecholamines in 24-hour urine (Noradrenaline, Adrenaline and Vanillylmandelic acid) for diagnosis of Phaeochromocytomas.

Table 9: eage 47> Sensitivity and Specificity of tests for Phaeochromocytoma

Sensitivity and Specificity of various procedures for Diagnosis of Phaeochromocytoma (headings) Procedures – Sensitivity – Specificity (and under Procedures) Sonography – Computer tomography – Magnetic Resonance Imaging – MIBG-Scintigraphy – Adrenaline in 24-hour Urine – Noradrenaline in 24-hour urine – Vanillylmandelic acid in 24-hour urine – Adrenaline in plasma – Noradrenaline in plasma

2. Prevalence of VHL with Phaeochromocytomas

To answer this question the author collected a registry of 225 patients with symptomatic Phaeochromocytomas. cpage 48> Through 1993, the patients were exclusively in the clinical program. After 1994 they were also DNA-tested. A large blood testing bank was created and cases from the years prior to 1994 were included in the bank. On the whole 26% of the Phaeochromocytoma proved to be manifestations of the VHL. The mutations, which were detected in the VHL-associated Phaeochromocytomas, are illustrated in Table 10. This shows that predominantly the Missense Mutation predisposes to phaeochromocytomas. This contrasts sharply with the mutation spectrum for retinal angiomas, for haemangioblastoma of the CNS and for renal carcinomas. The patients' collective information makes possible some assertions about important aspects:

Table 10: cpage 48> Germline of VHL gene in patients with phaemochromocytomas. (Extracted from Table 2)

<page 49>

Of multiple Phaeochromocytomas (n=36) 78% were manifestations of VHL disease.

Of extra-adrenal Phaeochromocytomas (n=21) 70% were manifestations of VHL disease.

Of malignant Phaeochromocytomas (n=6) 1 case was a manifestation of VHL disease.

Of Phaeochromocytomas in children (n=23) 80% were manifestations of VHL disease.

Furthermore there were important regional differences, in the Südbaden (South Baden) region because of a so-called Founder Effect (30), 58% showed a high prevalence of phaeochromocytoma. This research finding is important, since through this additional VHL-associated tumor patients may be identified early, and the VHL disease itself may be detected and treated early among their relatives.

> **Figure 28:** <page 49> Precautionary findings in a 30-year-old VHL patient Multifocal Phaeochromocytomas. CT: 3 sections on the left, MIBG-Scintigraphs middle above, coronal MRI's middle below, transverse MRI 3 sections on the right.

3. Preventive diagnosis and therapy

The preventive diagnosis is for the relatives of VHL patients. These families are target groups of preventive diagnosis.

Carrier detection testing has a significant role to play in establishing the affect-carriers among the relatives of people with VHL. An example of multifocal Phaeochromcytomas detected in this way is shown in Figure 28.

Table 11: <page 50> Newly detected Phaeochromocytomas in VHL families Column headings = Case – Age – Sex – Location –Mmaximum size – Remarks : not operable, number of tumors.

<page 51>

Counting the tumors that had been found before the introduction of DNA diagnosis, the number of patients with preventively discovered phaeochromocytomas was 30 and the number of tumors in these 30 patients was 41 (Table 11).

<page 51> Adrenal-sparing Phaeochromocytoma surgery, Laparoscopic operation

The high risk for bilateral adrenal phaeochromocytoma resulted from the use of classical therapy, the Adrenalectomy leading to surgical Addison's Disease and therefore to a life-long requirement for substitution of Steroids. This causes the patient to need to take tablets daily. Because we ascertained such a very low incidence of malignant Phaeochromocytomas in VHL, we re-examined the question of the optimal therapy. The concept of organ-saving adrenal surgery in cases of Phaeochromocytomas was brought to the attention of the author in Germany for the first time during the course of this Project and has been the standard practice in the Freiburg University clinic since 1984. For this reason, for the 39 Phaeochromocytoma patients it could be shown that this method was feasible, and was successful in almost all cases (in 38 out of 39 cases). The success of the therapy was evaluated based on long term follow-up observations (average period of observation 6 years) (24). Because of the extremely rare bilateral adrenal Phaeochromocytomas being observed in sporadic Phaeochromocytomas, the group is made up of patients with familiar Phaeochromocytoma on the contralateral adrenal, has profited the most from the organ-saving adrenal surgery. In this connection, DNA diagnosis for relatives of patients with unilateral phaeochromocytoma to rule out VHL would be of special significance.

The introduction of the organ-sparing Phaeochromocytoma surgery is one example that in the course of the Project, new standards of therapy could be developed from the character of the model.

Another interesting modification of Phaeochromocytoma treatment is the laparoscopic resection of these tumors. This is especially suitable for patients, in whom through the preventive medical precautions these tumors were detected in a relatively asymptomatic state. The large scars caused by adrenal surgery, <page 52> and the postoperative discomfort of the scarring, can now be avoided (Fig. 29). Our first experiences came in the course of this Project with 3 patients, each having bilateral adrenal tumors and in addition extra-adrenal Phaeochromocytomas, on whom favorable clinical and cosmetically preferable results were obtained (37).

Figure 29: <page 52> Abdominal scars from adrenal surgery

Abdominal scars after laparotomy (above) and after laparoscopic tumor resection of multiple Phaeochromocytomas (below), same case as Fig. 28.

<page 52> Phaeochromocytoma as a unique VHL manifestation

It is an extremely important point that in the course of this Project it was firmly established that Phaeochromocytoma can be the sole manifestation of VHL. Altogether 48 patients with symptomatic or asymptomatic Phaeochromocytomas without other lesions of the VHL spectrum were registered, who exhibited the germline mutation of the VHL gene.

<page 53> This was especially impressive among 19 children registered (Table 12); only one of these children had an additional symptomatic lesion, in two others such lesions were diagnosed in asymptomatic stages by means of systematic clinical examinations.

Table 12: <page 53> Germline mutations in children with Phaeochromocytoma (*headings: Age – Sex – Mutation – Symptomatic associated lesion – Family history*) Nein = no, ja = yes

With thorough examinations, asymptomatic lesions were detected in two cases ("nein") and in three cases lesions in early stages were found in relatives. At least two cases proved to be new mutations.

<page 54> Pancreatic changes

In VHL, changes occur in exocrine as well as endocrine parts of the Pancreas.

1. Cystic Pancreatic changes

Cystic pancreatic changes are frequent in VHL. Frequently are found multiple serous cysts, variable in size dispersed over the entire organ (Fig.30). Some patients show the presence of one serous Cystic adenoma (Fig. 31). Other patients have only very discrete changes with few cysts. The finding should be documented in the preliminary examination by Computer Tomography or MRI, while for follow-up examinations sonography examinations seem sufficient. In a few cases, cystic pancreatic changes may cause slight to moderately severe abdominal pressure or even pain, because of the increase in size of the organ. Pancreatitis or an obstructive jaundice are rare occurrences.

Among the registered patients there were 23% cystic pancreas changes. Pain, which may demand therapy, was present in only one patient. In this case a percutaneous puncture was undertaken, introducing a catheter, draining fluid and attempting destruction of the cysts with a high percent alcohol. This treatment resulted in a small remaining cyst, and the pain was perceptibly reduced.

Figure 30: <page 54> Multiple Pancreatic cysts in VHL disease

Figure 31: <page 55> Serous cystic adenoma of the Pancreas. *Asymptomatic 75 year old patient*

More important than the therapy is the knowledge that pancreatic cysts occur in VHL. Mistaken differential diagnoses have included Echincoccus (tape-worm), postpancreatic cysts and the cysts of polycystic renal disease.

2. Islet cell tumors

Among the registered cases there are 6 patients with Islet cell tumors. These tumors appear infrequently in VHL rarely but they are still listed under the typical manifestations (51).

Figure 32: cpage 55> Islet cell tumor in a 20-year-old patient (incidental finding in a patient with VHL)

<page 56> Epididymal cystadenoma

Cystadenomas of the Epididymis are non-malignant tumors. They occur in about 14% of patients with VHL disease (2, 51). They were partially noted as chance findings. This occurs bilaterally from formations of an epididymal cystadenoma, so infertility may result due to Azoospermia. These tumors were observed in 8% of the registered males with VHL disease.

Figure 33: cpage 56> Testing for Epididymal cystadenoma
Sonographic and histologic findings in the case of Epididymal cystadenoma

<page 57> Inner Ear Tumors (endolymphatic sac tumor)

Inner ear tumors in VHL correspond to the International nomenclature endolymphatic sac tumor or ELST (Fig. 34). They arise from cells of the inner ear sacculus. Clinically, hearing noises (Tinnitus) and diminished hearing stand out as initial signs. Histologically, solid or follicular structures are found by means of which the confusion with metastasis of Thyroid carcinomas has been a problem (19). A recently published study from NIH shows ELST in more than 11% of their VHL patients (56). The diagnosis is determined using MRI or CT of the temporal bone, whereby the same picture-taking technique is used as for Haemangioblastomas of the CNS.

The VHL registry that forms the basis of this study includes only one case, a female presently 50 year old, operated for bilateral ELST in 1982 and 1984. The tumors were completely resected. She has been successfully supplied with a so-called Cochlear-Implant. Through this implant she regained good hearing (19).

An ELST should be resected as long as there is a possibility of saving the hearing, since the operation itself does not necessarily produce a hearing loss.

Figure 34: page 57> Location of the "Endolymphatic Sac"
(enlarged in inset)(25)

<page 58> Prevention of recurrences

Patients with VHL disease carry a life-long risk. Any of the tumors in the total lesion complex may develop. 32% of the registered patients had to be operated on more than once. The prevention of "relapse" deals therefore with a true relapse, that is tumors in the operated region, but also includes detection at an early stage of any new tumors in the treated organs or elsewhere in the organ system. To prevent relapse, early diagnosis must be integrated into a program in a meaningful way. The control studies must include the as yet unaffected organs in the interest of prevention. The author was intimately involved in discussions at international symposia regarding examination intervals and range of the control study program. Control examinations at yearly intervals were recommended and were required during the Project.

The general recommendations should be further modified, as two aspects are under consideration but have not yet been clarified:

1. The growth of the lesion should be characterized. To date only insufficient data exists. For VHL-associated renal carcinoma, the author has established an increase in diameter of 0.25 mm/year in 12 cases with an average observation period of 5 years (22). With retinal angiomas relapses are in a narrower sense, that is tumors in a Laser coagulated area, as well as new tumors in the form of microangiomas occurred after 1 $\frac{1}{2}$ years and then located and again coagulated without visual reduction (24).

2. The spectrum of involved organs and the aggressiveness of VHL is partly professed to be due to the existing germline mutations. Sufficient data has been obtained in the course of this Project about the Mutations nt.505 T \rightarrow C, nt.479 T \rightarrow C and nt. 775 C \rightarrow G. Data for other mutations are in progress through international co-operation. The problem presented here is discussed in the section *Genotype/Phenotype correlations*.

In the course of this Project, through the available control studies, the following relapses or new tumors were detected: Retinal angiomas in 11 patients, Haemangioblastomas of the CNS in 7 patients, Phaeochromocytomas in 9 patients.

In summary it is to be said that in the recommended programs relapses can be diagnosed in early stages and treated.

<page 59> Genotype/Phenotype correlations

Genotype/Phenotype correlations offer important information for VHL with regard to the lesions actually observed in patients with patricular mutations (11, 20, 31). The mutations identified in the course of the Project are shown again in Table 14. In this listing there is no consideration of the number of tumors, possible bilateral tumors in paired organs, locations of Haemangioblastomas in the head region, brain stem and spinal cord as well as the number of affect-carriers, into which the corresponding lesions have been grouped.

For the sake of the patients, their relatives who were newly identified as affect-carriers, and for the physicians involved, these lists of relevant lesions provided no useful benefit. They are an attempt to answer the question, which risks are tied to a specific mutation. To make confident assessments of this, a great deal of data is needed, which up to now has been very difficult to gather. This involves the factors of age, sex, symptomatic or asymptomatic lesions, quality of clinical examinations and number of known carriers of identical mutation.

For the mutation nt 479 C \rightarrow T the informative materials are grouped in Table 15. The information is fragmentary, although the data presented may serve as a basis for compiling a Risk profile.

For mutation nt 775 C \rightarrow G the risk profile was published (30), it is reproduced in Table 16. In spite of careful research and studies, the information is full of gaps. It shows that the penetrance is high, highest for Phaeochromocytoma. Unusual situations occurred in two patients, medullary thyroid carcinoma which were differentially diagnosed. The demarcation was difficult in comparison with another hereditary tumor syndrome, the multiple endocrine neoplasia Type 2.

Furthermore it was noted that the names given the lesions of the retinal angioma and the haemangioblastoma of the CNS were completely lacking.

Only for the mutation nt 505 T \rightarrow C, can prospective studies now be carried out on a large number of affect-carriers (the author, presently unpublished). The essential data are reproduced in Fig. 35. The age-dependant penetrance is noted for occasional lesions. This penetrance is high for Phaeochromocytomas and retinal angiomas, low for Haemangioblastomas of the CNS, and other lesions of the VHL complex are rarities in this mutation. Hence this offers an almost ideal possibility for a consultation service for risk profiles and prognoses.

Table 13: there is no table 13 in the original

Table 14: <page 60> Germline mutations and associated lesions

Germline mutations of the VHL gene and lesions associated with each mutation. (at bottom of page 61 – the key) Zahl = patients (n – number), RA = Retinal angioma, Hbl = Haemangioblastoma of the CNS, NZ = Renal cyst, NT = Renal tumor, Ph = Phaeochromocytoma, Pz = Pancreatic cyst, IT = Islet cell tumor, NH = Epididymal cystadenoma, E = ELST. Abbreviations of column 2 see table 2. The numbers always indicate how many of the affect-carriers develop each lesion.

Table 15: <page 62> Findings and risk profile for patients with germline mutation nt. 479 T \rightarrow C

Columns: Case, Age and Sex – Kidney – Head – Spinal cord – Eye – Pancreas – Adrenal, Paraganglia

Table 16: <page 62> Findings and risk profile for patients with germline mutation nt. 775 C \rightarrow T

Columns: Case, Age, Sex – Eye – Head – Spinal cord – Kidney – Pancreas – Adrenal, Paraganglia – Thyroid carcinoma

Figure 35: <page 63> Penetrance –Calculations for mutation VHL nt. 505 T \rightarrow C

<page 64> Cost analysis

The actual costs are given here with the 2.5 times the scale of charges for physicians of 1996. Follow-up examinations occurred in the course of the check-up programs as clinical examinations or DNA examinations:

G	erman Marks	Euro*	USD*
MRI's of the head with Gadolinium	1540	1370	\$747.57
MRI's of the spinal cord with Gadolinium	1750	1557	\$849.52
MRI's of the abdomen	1540	1370	\$747.57
Ophthalmological examinations	42	37	\$20.39
Fluorescein Angiograms of the retina	138	123	\$66.99
Adrenaline, Noradrenaline, Vanillylmandelic acid in 24 hour Urin	ne 325	289	\$157.77
Isolation of nucleic acids	257	229	\$124.76
SSCP (PCR by primer pair)	314	279	\$152.43
DNA-sequencing	570	507	\$276.70
Restriction-digestion per enzyme	43	38	\$20.87
Hybridization per probe	363	323	\$176.21

*In July of 2000, 1 DM (German mark) = 0.89 € (Euro) = 0.48 USD (U.S. Dollars)

The basic clinical examination program, including MRI of the head and spinal cord, MRI of the abdomen, ophthalmological examination and Fluorescein angiogram of the retina, totaled about 5010 DM [4459ε , \$2432].

The DNA test with the use of a total of six primer pairs amounts to 1875 DM [1669€, \$910] or (in case the SSCP examination produces a Band-shift by omission of a hybridization and additional DNA sequencing) 2080 DM [1851€, \$1010]. For family dependants this amounted to about 570 DM [507€, \$277] per person. The DNA diagnosis therefore is more economical than the clinical examination program. This is certainly the first expense to be questioned in family screening, where one has to take into account that 50% of the relatives are expected to be primary grade affect-carriers. Therefore it appears mathematically, that for each of two relatives clinical examination would have been unnecessary, and that thus for one out of every two persons in a family, about 1260 DM [1121€, \$612] were saved.

<page 65> Legal aspects

There are special legal concerns for VHL patients, as well as for aymptomatic people with VHL germline mutations. There are risks of discrimination in the areas of insurance rights and employment rights. In the course of running the Project, knowledge regarding these concerns was also collected.

The medical insurance with legitimate insurance companies in Germany is not complicated. Entrance into a private medical insurance can of course be problematic. Considerable difficulties may arise with examinations for Life Insurance. The author was asked for an expert statement in such a case. With good treatment and good long-life prognoses – in a special case presenting a mutation nt. 505 C \rightarrow T (see *Genotype/Phenotype correlations*) – the conclusion was that the patient was granted life insurance under normal conditions.

The beginning of new employment can be associated with unexpected barriers. The status facing a VHL germline mutation carrier leads to the question whether he will have the probability of reaching retirement age. For VHL in general a definite improvement in prognosis has been made through modern diagnosis, modern therapy and, especially through the level of information available to the patient. Cases have arisen concerning employment in the civil service.

A VHL patient with Haemangioblastomas of the spinal cord and severe back pains was granted an early retirement, which had at first been denied.

To summarize is to say that in such situations, the data provided here and the general state of research must be used as arguments. Unfavorable consequences of prior decisions on new questions are still conceivable.

<page 66> National Networking

In a preventive medicine project the question of the ability to communicate with other institutions arises. In doing so the feasibility and need are to be separated. On the point of feasibility, clinical and DNA diagnoses and programs were based on standard procedures, so that the requirements for treatment at any large clinic were readily available. Essentially it still requires a background of experience. At Centers which are dealing with VHL -- such as in Freiburg, Bethesda, Cleveland, Paris and Utrecht -- this can be achieved, although country-specific conditions have presented problems. The insurance situation in the USA on the whole is frequently bad, and treatment at the National Institutes of Health is possible only for patients who are able to be fitted into special research programs.

In Germany, it appears that due to the up to now overlooked number of patients and the importance of background knowledge, a network is essential. Therefore already in Berlin, a group of Human Geneticists, Ophthalmologists, Surgeons, in cooperation with the author, has been established for advice and treatment. This Center has taken over the care of about 30 patients. It is also important to have a meeting of family physicians and specialists in the residential area of the patients, in order to establish control studies in that region. Documents can then be mailed to a more experienced center for consultation. It is only through interest in this Project, that satellite institutions can be developed. Collaborative discussion and shared responsibility are becoming increasingly important.

<page 67> Information service

Information about VHL should be given as specifically as possible to the occurring questions. This as a rule requires a discussion. The author has conducted a great many such discussions, primarily with patients and their relatives, but also with colleagues about diagnostic and therapeutic questions, or also about preparation for genetics consultations. As a rule, in one of the first consultations there are many aspects of importance, which will require a discussion period of at least 30 minutes. Experience indicates that the information will be occasionally only partially understood or even distorted.

For this reason, the author wrote in 1996 a comprehensive *Information Pamphlet*, which is provided free following the consultation or can even be mailed. This Information Pamphlet was written in German in a manner intelligible to everyone, and contained for the lay public and the physicians, all the information available at that time. The information pamphlet was circulated among patients on the Internet. (At http://www.hippel-lindau.de/, select "downloads".) As a result of this information exchange, a number of patients from inside and outside the country have contacted the author about specific problems and confirmed a good assimilation of the Information Pamphlet

The Project was repeatedly mentioned in the press, and also in the Nature and Science sections of the *Frankfurter Allgemeinen Zeitung* for Jan. 19, 1994, and May 2, 1996.

Information Evenings were offered by the author 1 - 2 times per year, at which special innovations were discussed or visiting colleagues came to speak. The acceptance of these efforts by the patients has been very favorable, and the information exchange, without citing actual individual problems, appears to be very important.

An formal Self-Help group has been formed at this time, in which the author has played an advisory role.

One such Self-Help group, the VHL Family Alliance, has existed for a few years in the USA. They produce a newsletter four times per year. The author was invited to serve on their Medical Advisory Board

and has done so for a long time. The VHL Family Alliance, since 1994, has organized annual patient/provider conferences, always a different State.

<page 68> Balance sheet and perspectives

Balance sheet

The preventive medicine Project for pre-care and post-care of patients with VHL and for patients with lesions in a multitude of forms, through interdisciplinary cooperation, has been built up over the course of 15 years. Through cooperative working with the patients, a body of relevant practical knowledge has been attained. Diagnosis and Therapy have spread nation-wide. A balance between individual opinions about possible organ-sparing procedures versus a waiting period, as well as open discussion about all aspects of this multifaceted disease, requies a basis of mutual trust. This atmosphere of cooperation and trust is a necessary ingredient for patients with the life-long burden of this severe disease.

From 1983 to 1998, the following operations were performed at the Freiburg University Clinic:

40 operations because of Haemangiomas of the CNS, 17 Whipple's operations because of Islet cell tumors and 3 resections of inner ear tumor, as well as Laser treatments on 66 eyes. This was in contrast to two neurosurgical complications (incomplete pareses), a surgical Addison disease and in one case extensive liver necrosis following Islet cell tumor removal. Through therapeutic or omitted precautions in the course of the Project, there were no instances of a new occurrence of loss of vision, an operation-related death, a metastasis from renal carcinomas or dialysis failure.

DNA examinations of the VHL gene for verification or exclusion of germline mutations were carried out on 810 persons.

The results of the molecular-genetic laboratories reveal practical relevant findings. The Project was able to show that it is importan, for VHL and other genetic diseases, to gather fundamental information, and especially to work closely between clinic and the molecular-genetics team. Thus the establishment and integration of the molecular-genetic laboratories with relevant clinic centers has proven to be especially favorable.

<page 69> Perspectives

It is essential that the data presented here, that has been acquired in the course of scientific inquiry into these questions, be elaborated and published. The problems of the affected individuals are still with them, regardless of their current situations, and problems exist as well in forthcoming generations. Every effort must be made to guarantee the long-term duration of the Project. This will require greater assurance of the availability for every individual of the life-long controls and sufficient time for consultations.

Necessary also is the expansion of the Project through the Network by linking as many large clinics as possible with the primary goal that all patients who are possibly at risk for VHL can be screened.

<page 69> New developments in the expansion of diagnoses

For molecular-genetic diagnosis it is necessary to improve the diagnosis in order to be able to determine the approximately 20% of germline mutations which have not yet been identified.

A diagnostic improvement would be the disclosure of the activity parameters of VHL, which could be measured by a blood test.

Corresponding expansions have not been fulfilled for erythropoietin and the vascular endothelial growth factor (VEGF) (26).

Medicines for reduction of the growth of VHL-associated tumors should be developed. Candidates for these are Pharmacologists with antiangiogenetic experience.

The molecular-genetic research of the true tumor genesis is for VHL in a hopeful state. The possibilities of suppressing tumorigenesis or the growth of tumors by interference with the molecular-genetic mechanisms seems as yet to lie in the distant future.

<page 70> Registry

The total Project actually embraced the following groups of persons: 1. Patients with retinal angiomas 124 2. Patients with Haemangioblastomas of the CNS 257 3. Patients with Renal carcinomas 547 4. Patients with Phaeochromocytomas 225 5. Patients with Islet cell tumors 6 6. Control person for molecular-genetic findings 300 7. Patients with VHL disease 327 8. Relatives with exclusion of VHL disease 200

The preceding groups of persons overlapped partially. That numbers given represent the state of the register on 1 March 1998.

<page 70> Additional aspects of the Project

The following reference list represents the important works of authors for preventive medicine questions about VHL (citations 1-37). Papers not relevant to preventive medicine are not included here. Following those are the authors who have presented general seminar papers at international congresses, national congresses and at hospitals.

Up to now, five Information Evenings have been organized and held especially for VHL Patients in Germany and the German-speaking countries.

Since 1994, the author has participated in conferences, in which the preventive medicine for VHL was part of the program. Some of these conferences were held with the inclusion of patients, for example, the Patient/Provider conferences in Kansas City, Kansas; Burlington, Massachusetts; Honolulu, Hawaii; and Bethesda, Maryland.

Since 1994, the author has been active on the Medical Advisory Board of the American Self-Help group the VHL Family Alliance.

<page 71> References

Additional copies of the original German monograph are available from:

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